ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.17

Volume 130, pp. 17–25 *January–March 2015*

Dentipellicula austroafricana sp. nov. supported by morphological and phylogenetic analyses

Jia-Jia Chen[#], Lu-Lu Shen[#] & Yu-Cheng Dai[*]

Institute of Microbiology, Beijing Forestry University, P.O. Box 61, Beijing 100083, China

*Correspondence to: *daiyucheng2013@gmail.com*

Abstract — *Dentipellicula austroafricana* sp. nov. is described and illustrated from South Africa based on morphological characters and rDNA sequence data. It is characterized by an annual growth habit, resupinate basidiocarps, dense soft spines, a monomitic hyphal structure with non-amyloid, non-dextrinoid and acyanophilous generative hyphae, absence of cystidia, presence of cystidioles, and tiny rough basidiospores (2.4–2.9 × 2–2.2 µm). A molecular study based on the combined ITS (internal transcribed spacer region) and nLSU (the large nuclear ribosomal RNA subunit) dataset supports the new species in *Dentipellicula*. A key to accepted species of *Dentipellicula* is provided.

Key words — *Basidiomycota, Hericiaceae*, hydnoid fungi, *Russulales,* taxonomy, wood-inhabiting fungi

Introduction

Dentipellis Donk (*Russulales, Basidiomycota*), typified by *D. fragilis* (Pers.) Donk, was introduced for species characterized by an annual growth habit, hydnoid basidiocarps, soft spines, a monomitic hyphal structure with clamp connections and cyanophilous hyphae, and amyloid rough basidiospores (Dai et al. 2009, Ginns 1986, Zhou & Dai 2013). Zhou & Dai (2013), who demonstrated that *Dentipellis* was polyphyletic, segregated *Dentipellis leptodon* (Mont.) Maas Geest. and *Dentipellis taiwaniana* Sheng H. Wu from *Dentipellis* based on ITS and nLSU rDNA sequences. They proposed *Dentipellicula* Y.C. Dai & L.W. Zhou as a new genus for these two hydnoid fungal species, which are distinguished from *Dentipellis* by their lack of cyanophilous hyphae. *Dentipellicula taiwaniana* (Sheng H. Wu) Y.C. Dai & L.W. Zhou (the generic type), and *D. leptodon* (Mont.) Y.C. Dai & L.W. Zhou currently comprise the entire genus.

[#] Jia-Jia Chen and Lu-Lu Shen contributed equally to this work and share first-author status.

During studies on the hydnoid fungi, an undescribed *Dentipellicula* species from South Africa was identified based on morphological characters and phylogenetic analysis of ITS and nLSU sequences. We provide an illustrated description and include an identification key to all three species representing *Dentipellicula*.

Materials & methods

Morphological studies

Sections were studied microscopically according to Dai et al. (2010) at magnifications ≤1000× using a Nikon Eclipse 80i microscope with phase contrast illumination. Drawings were made with the aid of a drawing tube. Microscopic features, measurements, and drawings were made from sections stained with Cotton Blue and Melzer's reagent. Spores were measured from sections cut from the tubes. To present spore size variation, the 5% of measurements excluded from each end of the range are given in parentheses. Basidiospore spine lengths were not included in the measurements. Abbreviations include IKI = Melzer's reagent, IKI– = negative in Melzer's reagent, KOH = 5% potassium hydroxide, CB = Cotton Blue, CB+ = cyanophilous, CB– = acyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = the L/W ratio, and n = number of spores measured from given number of specimens. Special color terms follow Petersen (1996). The studied specimens are deposited in the herbaria as cited below; collection abbreviations follow Thiers (2014).

DNA extraction and sequencing

A CTAB rapid plant genome extraction kit (Aidlab Biotechnologies, Beijing) was used to obtain PCR products from dried specimens, according to the manufacturer's instructions with some modifications (Chen & Cui 2014). The DNA was amplified with the primers: ITS5 and ITS4 for ITS (White et al. 1990), and LR0R and LR7 for nLSU (Vilgalys & Hester 1990). The PCR procedure for ITS was as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 40 s, 54°C for 45 s and 72°C for 1 min, and a final extension of 72°C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 30 s, 50°C for 1 min and 72°C for 1.5 min, and a final extension of 72°C for 10 min. The PCR products were purified and sequenced in Beijing Genomics Institute, China with the same primers.

Phylogenetic analysis

New sequences, deposited in GenBank (Table 1), were aligned with additional sequences retrieved from GenBank (Table 1) using BioEdit (Hall 1999) and ClustalX (Thompson et al. 1997). *Bondarzewia podocarpi* Y.C. Dai & B.K. Cui and *B.* sp. were used as outgroup (Zhou & Dai 2013). Prior to phylogenetic analysis, ambiguous regions at the start and the end of the alignment were deleted and gaps were manually adjusted to optimize the alignment. The edited alignment was deposited at TreeBase (http://purl.org/phylo/treebase; submission ID 15859).

Table 1. Specimens used in ITS and nLSU sequence analyses.

Species	Sample no.	Locality	GenBank accession no.	
			ITS	nLSU
B. podocarpi	Dai 9261	China	KJ583207	KJ583221
Bondarzewia sp.	DAOM F-415	Canada	DQ200923	DQ234539
Dentipellicula austroafricana	**Dai 12580**	**South Africa**	**KJ855274**	**KJ855275**
D. leptodon	GB 011123	Uganda	EU118625	EU118625
D. taiwaniana	Dai 10867	China	JQ349115	JQ349101
	Cui 8346	China	JQ349114	JQ349100
Dentipellis coniferarum	Cui 10063	China	JQ349106	JQ349092
	Yuan 5623	China	JQ349107	JQ349093
D. dissita	NH 6280	Canada	AF506386	AF506386
D. fragilis	Dai 12550	China	JQ349110	JQ349096
	Dai 9009	China	JQ349108	JQ349094
D. microspora	Cui 10035	China	JQ349112	JQ349098
D. parmastoi	Cui 8513	China	JQ349113	JQ349099
Hericium abietis	NH 6990	Canada	AF506456	AF506456
H. alpestre	NH 13240	Russia	AF506457	AF506457
H. americanum	DAOM F-21467	Canada	AF506458	AF506458
H. cirrhatum	Tübingen F794	Germany	AF506385	AF506385
H. coralloides	NH 282	Sweden	AF506459	AF506459
H. erinaceus	NH 12163	Russia	AF506460	AF506460
Laxitextum bicolor	NH 5166	Sweden	AF310102	AF310102

Phylogenetic analysis followed Li & Cui (2013). Maximum parsimony (MP) analysis was performed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each maximum parsimonious tree (MPT) generated. Phylogenetic trees were visualized using Treeview (Page 1996).

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model of the combined dataset for Bayesian inference (BI). BI was calculated with MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) with a general time reversible (GTR)

model of DNA substitution and an invgamma distribution rate variation across sites. Four Markov chains were performed for 2 runs from random starting trees for 1 million generations of the combined ITS and nLSU dataset, and trees were sampled every 100 generations. The burn-in was set to discard the first 25% of the trees. A majority rule consensus tree of all remaining trees was calculated. Nodes that received BT support ≥75% and Bayesian posterior probabilities (BPP) ≥0.95 were considered as significantly supported.

To determine if the dataset was significantly conflicted, the partition homogeneity test option in PAUP 4.0b was used between the loci using 1000 replicates and the heuristic general search option. This test randomly shuffles phylogenetically informative sites between two paired loci: if the dataset is compatible, shuffling sites between the loci should not produce summed tree lengths that are significant greater than those produced by the observed data (Farris et al. 1995; Huelsenbeck et al. 1996).

Molecular phylogeny

Partition homogeneity test showed no conflicts for the two-gene combined loci (P ≥0.01). Therefore, in this study, ITS and nLSU were combined into a single analysis. The combined ITS and nLSU dataset included sequences from 20 fungal collections representing 17 species. The dataset had an aligned length of 1720 characters, of which 1246 characters are constant, 131 are variable and parsimony-uninformative, and 343 are parsimony-informative. MP analysis yielded 2 equally parsimonious trees (TL = 902, CI = 0. 708, RI = 0.786, RC = 0.557, HI = 0.292). The best model for the combined ITS and nLSU sequences dataset estimated and applied in the BI was GTR+I+G. BI resulted in a similar topology with an average standard deviation of split frequencies = 0.005368 to MP analysis, and thus only the MP tree was provided. Both BT values (≥50%) and BPPs (≥0.95) are shown at the nodes (Fig. 1).

The newly sequenced specimen from South Africa was embedded in the lineage of *Dentipellicula* (100% MP and 1.00 BPPs) and was closely related to *D. taiwaniana*.

Taxonomy

Dentipellicula austroafricana Jia J. Chen, L.L. Shen & Y.C. Dai, **sp. nov.** Fig. 2

MycoBank MB 811108

Differs from other *Dentipellicula* species by its exclusively resupinate basidiocarps, dense spines, absence of gloeoplerous hyphae and cystidia, presence of cystidioles, and tiny rough basidiospores.

Type — South Africa, KwaZulu-Natal Province, Durban, on charred wood of *Ficus*, 1.X.2011, Dai 12580 (**holotype**, BJFC; **isotype**, IFP; GenBank KJ855274, KJ855275).

Etymology — *austroafricana* (Lat.): referring to South Africa.

Fruitbody — Basidiocarps annual, resupinate, inseparable, hard corky upon drying, up to 6 cm long, 2 cm wide, 3 mm thick at center, without odor or taste.

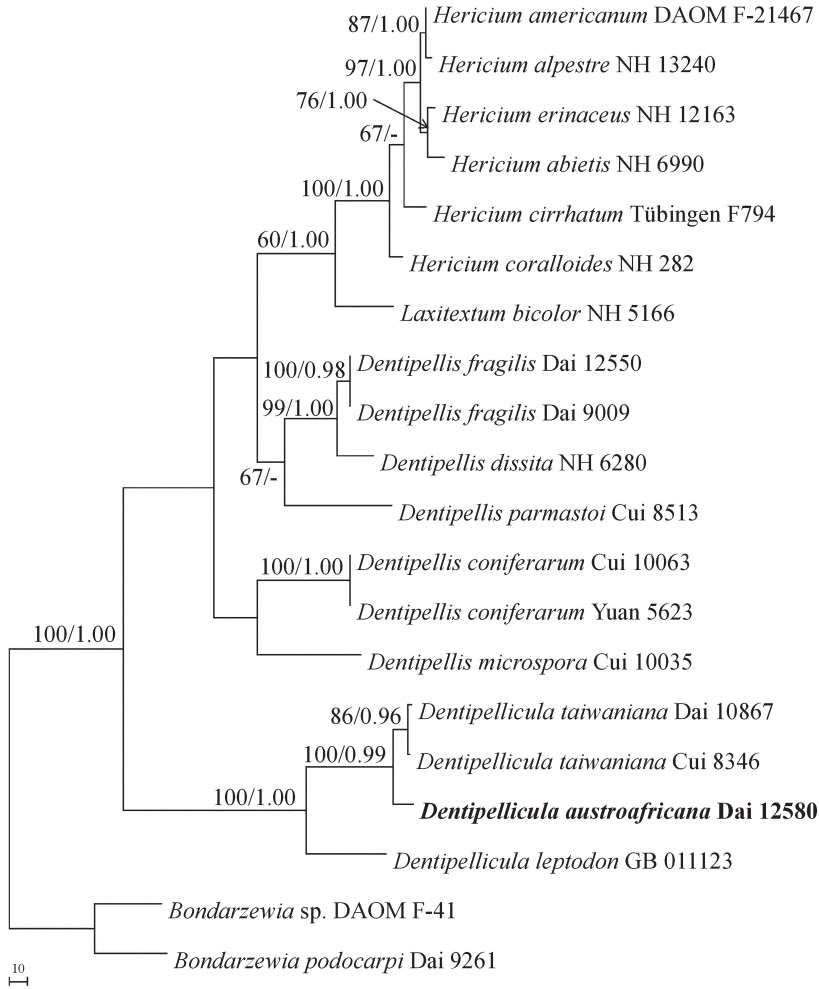

Fig. 1. Strict consensus tree illustrating the phylogenetic position of *Dentipellicula austroafricana*, generated by maximum parsimony method based on ITS+nLSU sequence data. Branches are labeled with parsimony bootstrap values ≥50% and Bayesian posterior probabilities ≥0.95.

Fresh spines soft, white to cream, when dry fragile, cream to buff, up to 2 mm long, 8–10 per mm across base. Margin cottony, buff to clay-buff, ≤1 mm wide. Subiculum very thin, soft corky, buff to reddish brown, ≤1 mm thick.

Hyphal structure — Hyphal system monomitic; generative hyphae with clamp connections, IKI–, CB–; tissues unchanged in KOH.

Subiculum — Generative hyphae colorless, thick-walled, frequently branched, interwoven, 4–6 µm in diam. Gloeoplerous hyphae absent.

Hymenophoral trama — Generative hyphae colorless, slightly thick-walled to thick-walled, moderately branched, more or less subparallel along the spines, 2–5.5 µm in diam. Gloeoplerous hyphae absent.

Hymenium — Cystidia absent; fusoid cystidioles present, hyaline, thin-walled, 16.5–18 × 1–4 µm; basidia clavate with four sterigmata and a basal clamp connection, 18–23 × 3.5–5 µm; basidioles similar to basidia in shape, but smaller than basidia. Basidiospores ellipsoid to broadly ellipsoid, thick-walled, colorless, minutely rough, strongly amyloid, CB+, 2.4–2.9(–3) × (1.8–)2–2.2 (–2.4) µm, L = 2.83 µm, W = 2.12 µm, Q = 1.29–1.39 (n = 60/1).

Other species examined — ***Dentipellicula leptodon*: CANADA. Ontario province, Algonquin Nat. Res.**, on *Betula*, 22.X.1966 (DAOM F-158439).

***Dentipellicula taiwaniana*: CHINA. Hainan province, Ledong County,** Jianfengling Nat. Res., on fallen angiosperm trunk, 12.V.2009, Dai 10872 (BJFC 5114).

Key to accepted species of *Dentipellicula*

1. Basidiospores >3.5 µm long . . . . . *D. leptodon*
1. Basidiospores <3.5 µm long 2
2. Spines 5–7 per mm; cystidia present . . . . . *D. austroafricana*
2. Spines 8–10 per mm; cystidia absent . . . . . *D. taiwaniana*

Discussion

Phylogenetically, *Dentipellicula austroafricana* is recognized in the *Dentipellicula* clade and distant from *Dentipellis, Hericium* Pers., and *Laxitextum* Lentz based on the combined ITS and nLSU sequences (Fig. 1). Morphologically, *D. austroafricana* is characterized by an annual growth habit, resupinate basidiocarps, soft and dense spines, a monomitic hyphal structure with non-dextrinoid generative hyphae, presence of cystidioles, and tiny rough basidiospores. Both morphology and rDNA sequence data confirmed that *D. austroafricana* is a new species in *Dentipellicula*.

According to the ITS and nLSU-based phylogeny (Fig. 1), *Dentipellicula austroafricana* is closely related to *D. taiwaniana,* which also has annual basidiocarps and soft spines but which is distinguished by its effused-reflexed basidiocarps, more scattered spines (5–7 per mm), narrower (2–3.5 µm) generative hyphae in the subiculum, presence of gloeocystidia, and longer basidiospores (2.8–3.4 × 2.1–2.4 µm; Dai et al. 2009, Wu 2007).

Dentipellicula leptodon also has annual basidiocarps and soft spines but differs from *D. austroafricana* in its narrower (2–4 µm) generative hyphae in the subiculum, presence of gloeopleurous hyphae in the subiculum and gloeocystidia, and larger basidiospores (3.6–4.2 × 2.8–3.3 µm; Dai et al. 2009,

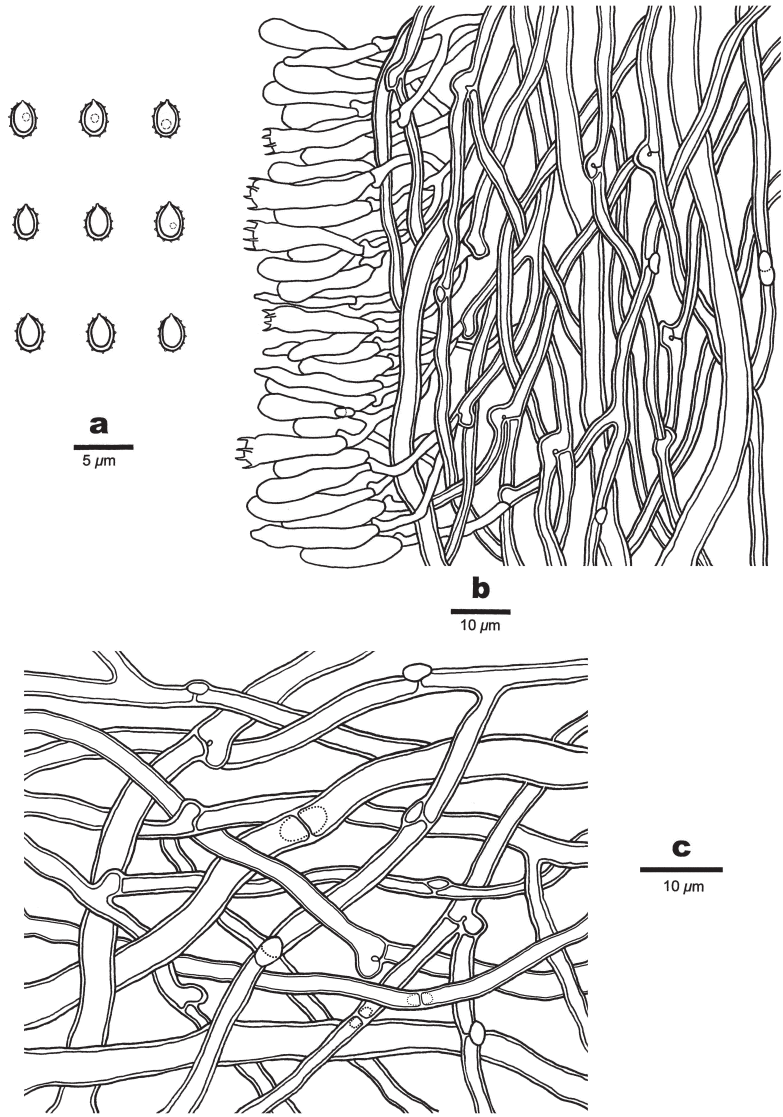

Fig. 2. *Dentipellicula austroafricana* (holotype).
a. Basidiospores. b. Vertical section through a spine. c. Hyphae from subiculum.

Ginns 1986). Moreover, the two species are separated in the ITS and nLSU-based phylogenetic analysis (Fig. 1).

Our phylogenetic tree was overall consistent with that presented by Zhou & Dai (2013): for now, *Dentipellis* remains polyphyletic based on ITS and nLSU rDNA sequences, while the new segregate genus *Dentipellicula* is monophyletic (Fig. 1). A fully resolved phylogeny for *Dentipellis* and its related genera requires evolutionary information from wider taxa samplings and more conserved gene markers.

Acknowledgements

We express our gratitude to Drs. Li-Wei Zhou (Institute of Applied Ecology, Chinese Academy of Sciences, China) and Michal Tomšovský (Mendel University in Brno, Czech Republic) who reviewed the manuscript. The research was financed by the Fundamental Research Funds for the Central Universities (No. BLYJ201403).

Literature cited

Chen JJ, Cui BK. 2014. *Phlebiporia bubalina* gen. et. sp. nov. (*Meruliaceae*, *Polyporales*) from southwest China with a preliminary phylogeny based on rDNA sequences. Mycol. Prog. 13: 563–573. http://dx.doi.org/10.1007/s11557-013-0940-4

Cui BK, Zhao CL, Dai YC. 2011. *Melanoderma microcarpum* gen. et sp. nov. (*Basidiomycota*) from China. Mycotaxon 116: 295–302. http://dx.doi.org/10.5248/116.295

Dai YC, Xiong HX, Wu SH. 2009. Notes on *Dentipellis* (*Russulales*, *Basidiomycota*). Mycosystema 28: 668–671.

Dai YC, Cui BK, Liu XY. 2010. *Bondarzewia podocarpi*, a new and remarkable polypore from tropical China. Mycologia 102: 881–886. http://dx.doi.org/10.3852/09-050

Farris JS, Källersjö M, Kluge AG, Bult C. 1995. Testing significance of incongruence. Cladistics 10: 315–319. http://dx.doi.org/10.1111/j.1096-0031.1994.tb00181.x

Felsenstein J. 1985. Confidence intervals on phylogenetics: an approach using bootstrap. Evolution 39: 783–791. http://dx.doi.org/10.2307/2408678

Ginns J. 1986. The genus *Dentipellis* (*Hericiaceae*). Windahlia 16: 35–45.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41: 95–98.

Huelsenbeck JP, Bull JJ, Cunningham E. 1996. Combining data in phylogenetic analysis. Trends Ecol. Evol. 11: 152–158. http://dx.doi.org/10.1016/0169-5347(96)10006-9

Li HJ, Cui BK. 2013. Taxonomy and phylogeny of the genus *Megasporoporia* and its related genera. Mycologia 105: 368–383. http://dx.doi.org/10.3852/12-114

Nylander JAA. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Page RDM. 1996. TreeView: application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. 12: 357–358.

Petersen JH. 1996. The Danish Mycological Society's colour-chart. Foreningen til Svampekundskabens Fremme, Greve. 6 p.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. http://dx.doi.org/10.1093/bioinformatics/btg180

Swofford DL. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sinauer Associates, Sunderland.

Thiers, B. 2014 [continuously updated]. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25: 4876–4882. http://dx.doi.org/10.1093/nar/25.24.4876

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. J. Bacteriol. 172: 4238–4246.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis et al. (eds).PCR Protocols: a guide to methods and applications. Academic Press, San Diego.

Wu SH. 2007. Three new species of corticioid fungi from Taiwan. Bot. Stud. 48: 325–330.

Zhou LW, Dai YC. 2013. Taxonomy and phylogeny of wood-inhabiting hydnoid species in *Russulales*: two new genera, three new species and two new combinations. Mycologia 105: 636–649. http://dx.doi.org/10.3852/12-011